

Stepwise Processing Analyses of the Single-Turnover PCSK9 Protease Reveal its Substrate Sequence Specificity and Link Clinical Genotype to Lipid Phenotype

John S. Chorba, Adri M. Galvan, Kevan M. Shokat

Supporting Information

1. Figure S-1
2. Figure S-2
3. Figure S-3
4. Figure S-4
5. Table S1

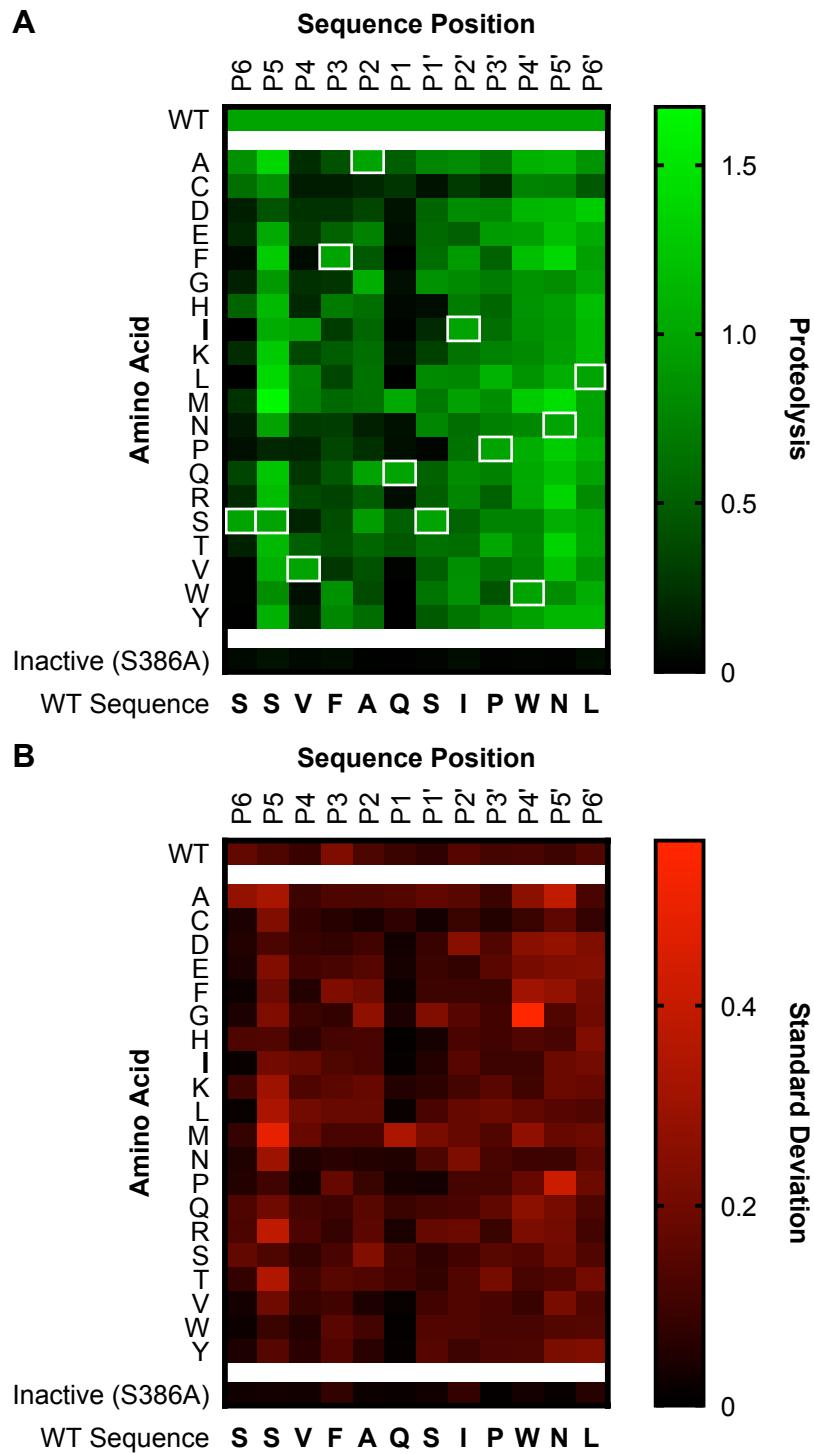


Figure S-1

A) Heatmap showing proteolytic activity for each single amino acid mutant in the P6 through P6' cleavage sequence. White rectangles illustrate the WT amino acid. A value of 0 (black) indicates essentially no proteolysis, and a value of +1 (green) indicates the WT value. B) Standard deviation of the heatmap values for proteolysis.

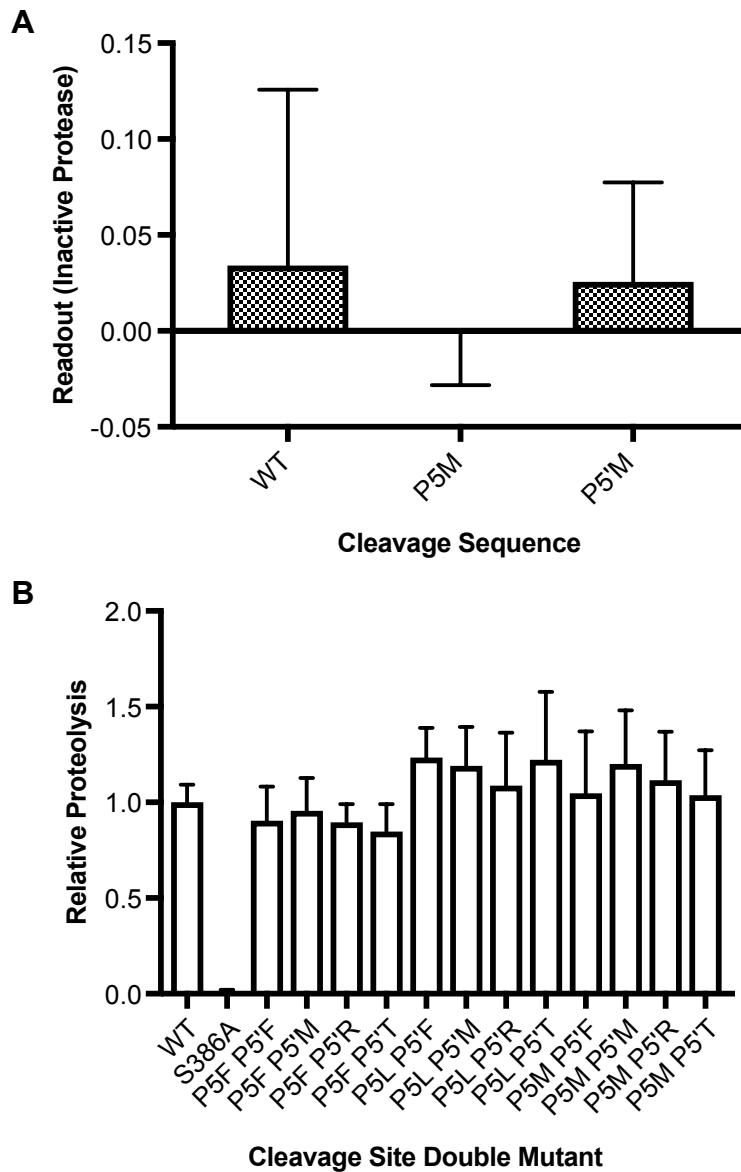


Figure S-2

A) Comparison of proteolytic activity of S386A (inactive) PCSK9 with selected methionine mutants in the cleavage sequence. Error bars indicate SD. B) Comparison of proteolytic activity on double mutant cleavage sequences. Error bars indicate SD.

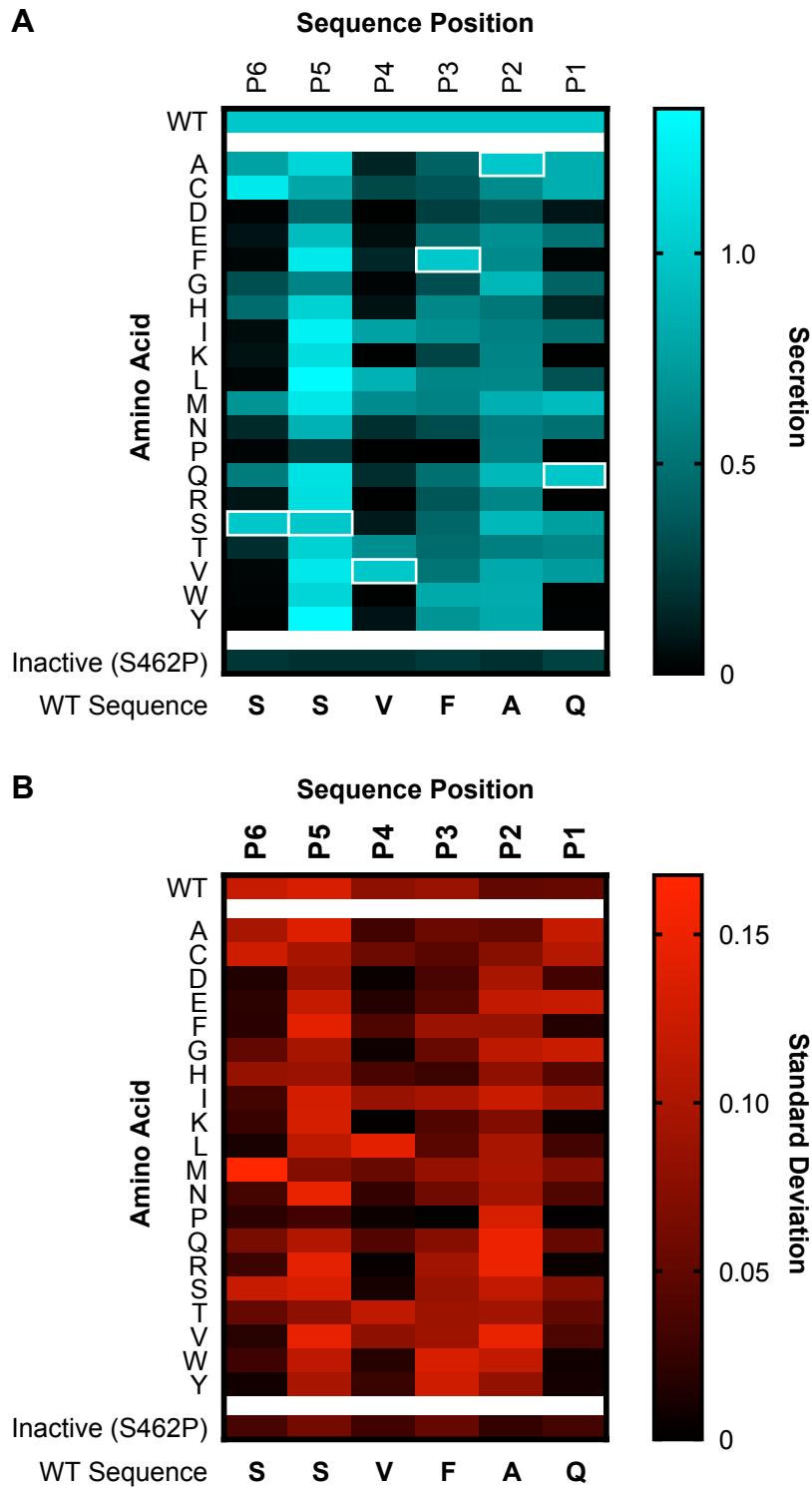


Figure S-3

A) Heatmap showing secretion activity for each single amino acid mutant in the P6 through P1 cleavage sequence. White rectangles illustrate the WT amino acid. A value of 0 (black) indicates essentially no secretion, and a value of +1 (cyan) indicates the WT value. B) Standard deviation of the heatmap values for secretion.

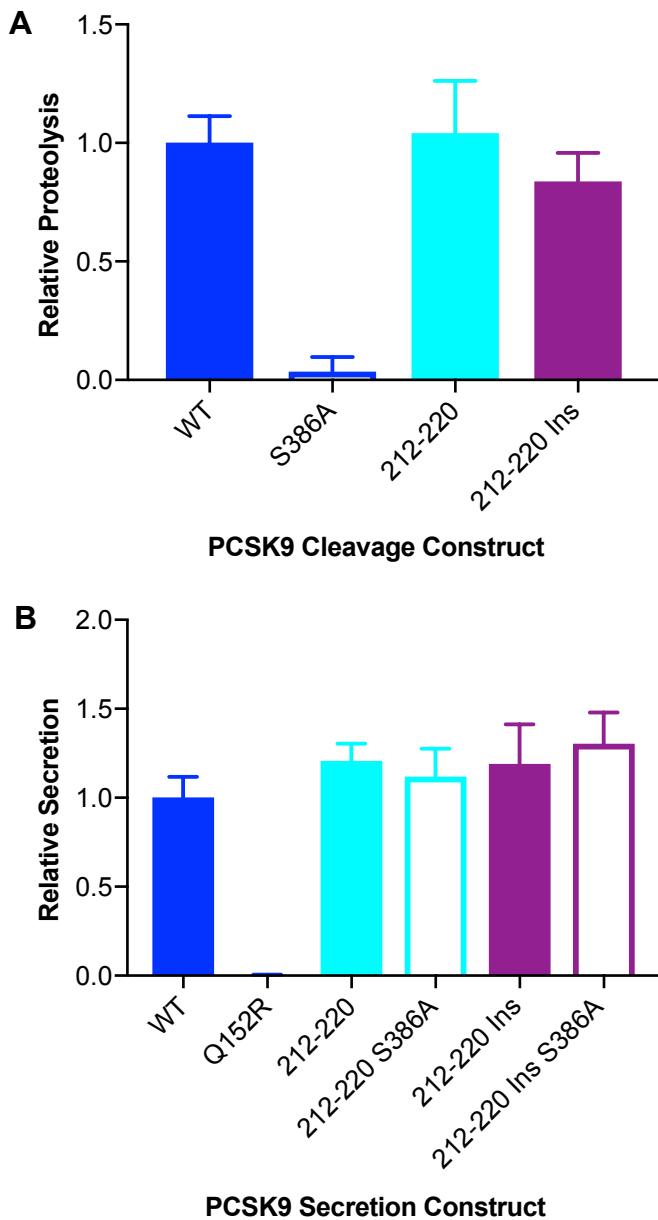


Figure S-4

A) Cleavage assay comparing relative cleavage activity of PCSK9 with the optimized cleavage sequence replacing the native GTRFHRQA sequence (212-220) or inserted just before the native GTRFHRQA sequence (212-220 Ins). Error bars indicate SD. B) *in trans* secretion assay comparing relative secretion of PCSK9 with the optimized cleavage sequence replacing the native GTRFHRQA sequence (212-220) or inserted just before the native GTRFHRQA sequence (212-220 Ins), with and without the S386A protease dead mutation. Error bars indicate SD.

Table S1: Statistically Significant Effects of SNPs on PCSK9 Processing

SNP	Cleavage	Secretion	Difference
E32K	0.2757		
D35Y			
R46L		-0.2729	
A53V	0.1884		
E54A			
E57K			
G59R	0.1906		
A62D	-0.592	-0.6269	
A68T			
T77I			
S89L	0.359		
R93C	-0.698	-0.4826	-0.2154
R104C	-0.6591	-0.8126	
G106R	-0.9196	-0.9364	
L108F	0.2873		
L108R	0.388	0.473	
V114A	-0.8576	-0.8996	
S127R	-0.6705	-0.6788	
D129G	-0.5466	-0.8517	0.3051
D129N		-0.4965	0.3915
Q152H	-0.8003	-0.8722	
N157K		-0.2326	0.322
A168E	0.2077	0.2264	
R194Q			
D204N	0.3444		
E206K	0.2236		
R215H	0.4301		
F216L	0.29		
R218S	0.3382		
Q219E	0.2907		
G236S	-0.2056	-0.7797	0.5741
R237W			
A239D			
A245T	0.6131		
R248H	0.3711		
L253F	-0.2514	-0.449	

G263S			
R272Q	0.3349		
P279T			
L283M			
R319W	-0.1734		
N354I	-0.8636	-0.9881	0.1245
R357H		-0.3096	
D374H			
D374Y		-0.2909	
F379A		-0.2861	
H391N		-0.3079	
H417Q			
I424V			
N425S			
R434W		-0.3429	0.3828
A443T			
G452D	0.2464		
S462P		-0.8477	0.8626
S465L		-0.4159	0.3911
P467A	-0.2682		
R469W			
V474I	0.3771		
E482G	0.5389	-0.3397	0.8786
R495Q	0.3701	-0.4354	0.8055
R496Q	0.2374		
R496W	0.2109		
N513D			0.335
A514T			
F515L	0.3352		
A522T	0.2698	<i>untested</i>	
H553R	0.4217	-0.5221	0.9438
Q554E	<i>untested</i>		
W566S	0.3251		
H591Q	0.3523		
P616L	0.5083		
Q619P	0.2089		
E620K			
V624M	0.4086		0.5881
V644I	0.678		

V650I	0.3317		
D651Y			
N652D	0.2087		
R659L			
D660N	0.2743		
S668G			
S668R			
E669K			
G670E			

Only statistically significant effects are noted. Untested SNPs are also noted.